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HELP for AML: Methylation Profiling Opens New Avenues

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There is growing evidence that aberrant gene expression in cancer is linked to epigenetic deregulation like promoter cytosine methylation in CpG-islands. In this issue of *Cancer Cell*, Figueroa et al. show that genome-wide promoter DNA methylation profiling reveals unique AML subgroups and methylation patterns that are associated with clinical outcome.

Acute myeloid leukemia (AML) represents a heterogeneous group of leukemias that differ with regard to biology, clinical course, and prognosis. During recent years, considerable progress has been made in deciphering chromosomal aberrations, gene mutations, and disordered gene expression that alter normal gene function (Figure 1). Based on this improved understanding of the molecular biology of AML, new diagnostic and prognostic markers have been defined (Dohner et al., 2009). Furthermore, novel therapeutic approaches are now being developed that target some of the molecular lesions, including epigenetic alterations by making use of, e.g., DNA methyltransferase inhibitors, although the extent and specific role of epigenetic modifications in leukemogenesis are still poorly understood.

Modulation of gene expression as a result of changes in histone and DNA modifications orchestrates key biological processes such as differentiation, imprinting, and silencing of large chromosomal domains. This type of control of gene expression is often termed epigenetics. It is becoming increasingly clear that a multitude of complex and interdependent epigenetic alterations collaborate with genetic changes in the development and progression of cancer (Jones and Baylin, 2007). Particularly, the importance of aberrant promoter cytosine methylation in CpG islands and the resulting gene silencing has been shown to be involved in cancer development. Improved insights into epigenetic control of gene expression may become especially valuable because genomic aberrations underlying cancer are largely irreversible, but epigenetic changes have the potential to be modulated for therapeutic benefit. Thus, a growing understanding of the linkage of genetic and epigenetic changes that promote leukemogenesis and the identification of appropriate biomarkers represents a prerequisite for successful epigenetics-directed leukemia therapy.

Following early reports that demonstrated hypermethylation of candidate tumor suppressor genes in hematologic malignancies, methylation-sensitive enzyme-based *r*estriction *l*andmark *g*enomic *s*canning (RLGS) was used as an initial tool for widespread assessment of altered DNA methylation. RLGS was used to detect genes with altered patterns of methylation in chemotherapy-sensitive, primary refractory and relapsed AML (Plass et al., 1999). Since then, novel technologies, such as microarray-based approaches, have provided increasingly powerful means for genome-wide quantitative investigation of DNA-methylation status in leukemia, suggesting that these approaches may identify novel disease markers (Gebhard et al., 2006).

In this issue of *Cancer Cell*, Figueroa et al. (2010) apply genome-wide promoter DNA methylation profiling to a large cohort of 344 newly diagnosed primary AML samples using the recently developed HELP (*H*paII tiny fragment *e*nrichment by *l*igation-mediated *P*CR) assay. Following data normalization and filtering, unsupervised hierarchical clustering analysis revealed 16 distinct methylation patterns that correlate well with known cytogenetically defined leukemias, including t(15;17), inv(16), t(8;21), or t(11q23) AML subgroups. This finding was perhaps predictable given each subset is characterized by a distinct gene-expression profile and the fusion oncogenes themselves may directly influence the epigenetic machinery in hematopoietic cells, but it is an important validation of the technology and the underlying assumption that genome-wide DNA methylation profiles will provide valuable insight.

Next, the authors provide valuable new insight into AML biology by integrating DNA methylation and gene expression to discover newly identified, not otherwise classified AML methylation subgroups. In line with a previous report (Gebhard et al., 2006), there was only a partial overlap of the aberrantly methylated and expressed genes in each cluster (Figueroa et al., 2010). Figueroa and colleagues point out that this may be in part due to silencing of critical genes that lead to widespread changes in gene expression. Furthermore, the fact that not all methylated genes are repressed at the expression level suggests that (1) aberrant gene expression in leukemia is influenced by many factors in addition to DNA methylation and (2) promoter DNA-methylation-associated transcriptional repression can either be overcome by other mechanisms or is active only at certain promoters. Interestingly, the concordantly regulated genes in each subgroup were significantly enriched for members of distinct pathways, such as p53 signaling and DNA-damage response, thereby

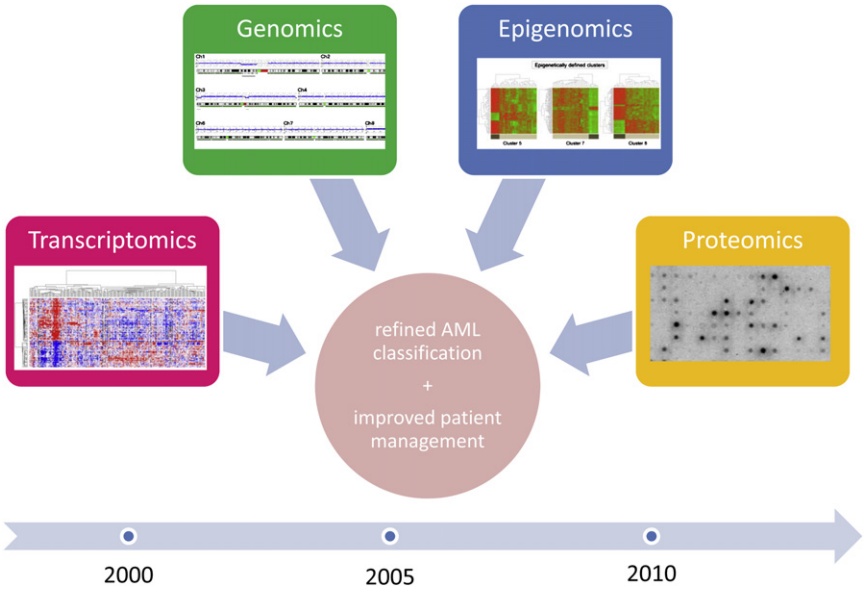

Figure 1. A Decade of "-omics" Research in AML
At the beginning of this decade, microarray-based gene expression profiling studies began providing new insight into leukemia pathogenesis. Since then, novel SNP microarray and sequencing-based genomics studies have unraveled novel findings, and now, genome-wide epigenomics promises to provide a deeper understanding of leukemia. Proteomics approaches are currently also under development (picture kindly provided by Drs. F. Heidel and T. Fischer, University of Magdeburg, Germany). Future efforts will aim toward integrated data analyses, thereby leading to a more comprehensive view of the biology of this disease, a prerequisite for refined AML classification and improved patient management.

pointing to distinct biological processes underlying the respective methylation-defined clusters that can now be assessed experimentally.

Strikingly, as there is known molecular heterogeneity with regard to secondary molecular aberrations influencing signaling pathways, such as *CBL*, *FLT3*, *JAK2*, *KIT*, *KRAS*, and *NRAS* mutations in core-binding factor leukemias, the relative uniformity of DNA-methylation profiles in this leukemia subset suggests the signaling mutations do not have a dominant impact on DNA methylation profiles. However, the primary genetic events contributing to impaired hematopoietic differentiation as defined by the WHO subgroup "AML with recurrent genetic abnormalities," including *NPM1* or *CEBPA* gene mutations, seem to have the greatest impact on the epigenetic profiles. In accordance, Figueroa and colleagues could not define a methylation pattern associated with *FLT3* aberrations (*FLT3*-ITD) (Figueroa et al., 2010). Thus, the newly discovered, methylation-pattern-defined AML subgroups might lead the way to yet unknown leukemia-"initiating" events. For example, does the cytogenetic heterogeneity in the newly identified cluster 2 reflect an epigenomic profile associated with a yet unknown mutation, or do multiple mutations result in the deregulation of an identical pathomechanism, thereby leading to a unique DNA methylation profile?

Similarly, the presence of a DNA methylation pattern "common" to most AML cases raises interesting issues: first, does leukemogenesis require silencing of a common set of genes, which are targeted by different primary events? Second, is this common methylation pattern caused by a leukemia-permissive event, that is common to all leukemia subgroups? Or third, does this reflect a remainder of the cell of origin methylation profile in which the leukemic transformation occurred? Finally, similar to a recent study that used MALDI-TOF matrix-assisted laser desorption/ionization time-of-flight mass-spectrometry to quantitatively screen the DNA-methylation status of 92 genomic regions in 256 AML samples and define a methylation-based outcome predictor for patient survival (Bullinger et al., 2009), the authors could build a robust DNA-methylation-based outcome predictor that provided novel information independent from known genomic markers (Figueroa et al., 2010). These large scale epigenetic analyses in AML support the use of genomic methylation markers for improved molecular classification and prognostication in adult AML.

The studies performed by Figueroa et al. provide an important initial glimpse into genome-wide DNA methylation profiles in AML through assessment of CpG island promoter methylation. Future investigations will assess whether regions of DNA methylation beyond promoter-associated CpG islands are important for disease. Recent genome-wide next generation sequencing studies demonstrated that methylation of CpG sites represent dynamic epigenetic marks that undergo extensive changes during cellular differentiation, particularly in regulatory regions outside of core promoters (Meissner et al., 2008), and initial single-base-resolution maps of DNA methylation demonstrate that methylation in non-CpG contexts might play an important role in undifferentiated cells (Lister et al., 2009). Furthermore, assessment of other chromatin modifications that play a role in leukemia development will likely provide insight (Neff and Armstrong, 2009). Foremost among these are the covalent histone modifications that can control gene activity as, e.g., ectopic histone methylation of specific lysine residues which are associated with MLL-fusion driven gene expression in leukemia (Krivtsov et al., 2008). The study by Figueroa and colleagues provides an optimal starting point for future efforts that will aim to extend epigenetic analysis in AML. This line of studies will further clarify the mechanisms underlying deregulated gene expression in leukemia and further refine prognostic markers which should result in improved patient management and outcome.

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All You Need Is a Mir-acle: The Role of Nontranslated RNAs in the Suppression of B Cell Chronic Lymphocytic Leukemia

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miR-15a and miR-16-1 were the first microRNAs linked to cancer because their genes are commonly deleted in human chronic lymphocytic leukemia (CLL). In this issue of *Cancer Cell*, Klein and coworkers show that deleting a region with these genes in mouse provides a faithful model for human CLL.

Chronic lymphocytic leukemia is the most frequent leukemia of adults in the Western world. Loss of 13q14.3 distal to the retinoblastoma locus is the most common chromosome aberration in CLL, which is presented in the majority of cases (Döhner et al., 2000). Studies of clonal evolution in CLL indicated that heterozygous deletion of 13q14 is an early event, whereas deletion of the second copy of this region occurs at a later time point at a lower frequency (Stilgenbauer et al., 2007). Mutation analysis of protein-coding genes in this region revealed no inactivation of candidate genes. However, a complex epigenetic regulatory tumor-suppressor mechanism that would control the expression of the entire region and would account also for cases without 13q14 deletion has been proposed (Mertens et al., 2006). Deletions at 13q14 also occur at high frequencies in other lymphomas and solid tumors, such as mantle cell lymphoma, multiple myeloma, and carcinoma of the prostate and the lung, suggesting a major tumor-suppressor mechanism mediated by this chromosome region.

Calin and coworkers were the first to show that 13q14 deletion in CLL is associated with downregulation of miR-15a and miR-16-1, whose genes cluster in the minimally deleted region (MDR) within 13q14 (Calin et al., 2002). This was the very first link between miRNAs and cancer. Because each miRNA is expected to regulate the expression of hundreds of different genes, several studies have been carried out to identify the targets of miR-15a and miR-16-1 (e.g., Calin et al., 2008). Because the current algorithms for predicting targets via sequence similarities are imperfect and the effects of miRNA level changes measured in vitro are highly dependent on cell systems used, the physiological relevance of some of the published targets remains controversial.

Klein and coworkers now report on the conclusive functional test of relevant 13q14 genes in mouse models (Klein et al., 2010). The MDR in 13q14 contains the noncoding RNA gene *DLEU2* with the miR-15a and miR-16-1 cluster in its intron 4. Klein and coworkers generated sophisticated mouse models that have either deletion of DLEU2 together with both miRNA genes (MDR deleted) or deletion of the two miRNA genes only. After 15 to 18 months, about 5% of the animals displayed monoclonal B cell lymphocytosis (MBL), which is a possible precursor to CLL. More importantly, 1/5 of the MDR-deleted and 1/8 of the miR-15a/16-1-deleted mice developed CLL or the related small cell lymphocytic leukemia. In addition, 9% of the MDR-deleted and 2% of the miR-15a/16-1-deleted animals developed a phenotype reminiscent of human diffuse large B cell lymphoma (DLBCL), a disease known to progress from CLL at low frequency. Thus, the deletion of the MDR caused B cell lymphoproliferative disorders, nicely recapitulating the spectrum of human CLL phenotypes.

Notably, the MDR-deleted mice died significantly earlier than did their wild-type littermates whereas miR-15a/16-1 deletion alone did not result in survival differences. Thus, although both MDR-deleted and miR-15/16-deleted mice develop an indolent disease reminiscent of CLL, there is at least one genetic element within the MDR other than miR-15a/16-1 that modulates the aggressiveness of the disease. DLEU2 and the first exon of DLEU1 are the only known